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# HIGH PRODUCTION VOLUME (HPV) CHEMICAL CHALLENGE PROGRAM

## REVISED TEST PLAN

For

## CASHEW NUT SHELL LIQUID

CAS No. 8007-24-7



Submitted to the US EPA

By

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Table of Contents

**Test Plan for Cashew Nut Shell Liquid**

1. Introduction

    1.1 Composition

    1.2 Commercial applications

    1.3 Worker/consumer exposure

2. Rationale for Selection of Compound for testing

3. Review of Existing Data and Development of Test Plan

    A. Evaluation of Existing Physicochemical Data and Proposed Testing

    B. Evaluation of Existing Environmental Fate Data and Proposed Testing

    C. Evaluation of Existing Ecotoxicity Data and Proposed Testing

    D. Evaluation of Existing Human Health Effects Data and Proposed Testing

4. Evaluation of Data for Quality and Acceptability

5. References

6. Robust Summaries

## 1. Introduction

Cashew nut shell liquid (CNSL) is one of the sources of naturally occurring phenols. It is obtained from the shell of a cashew nut. About 30-35% CNSL is present in the shell, which amounts to approximately 67% of the nut.

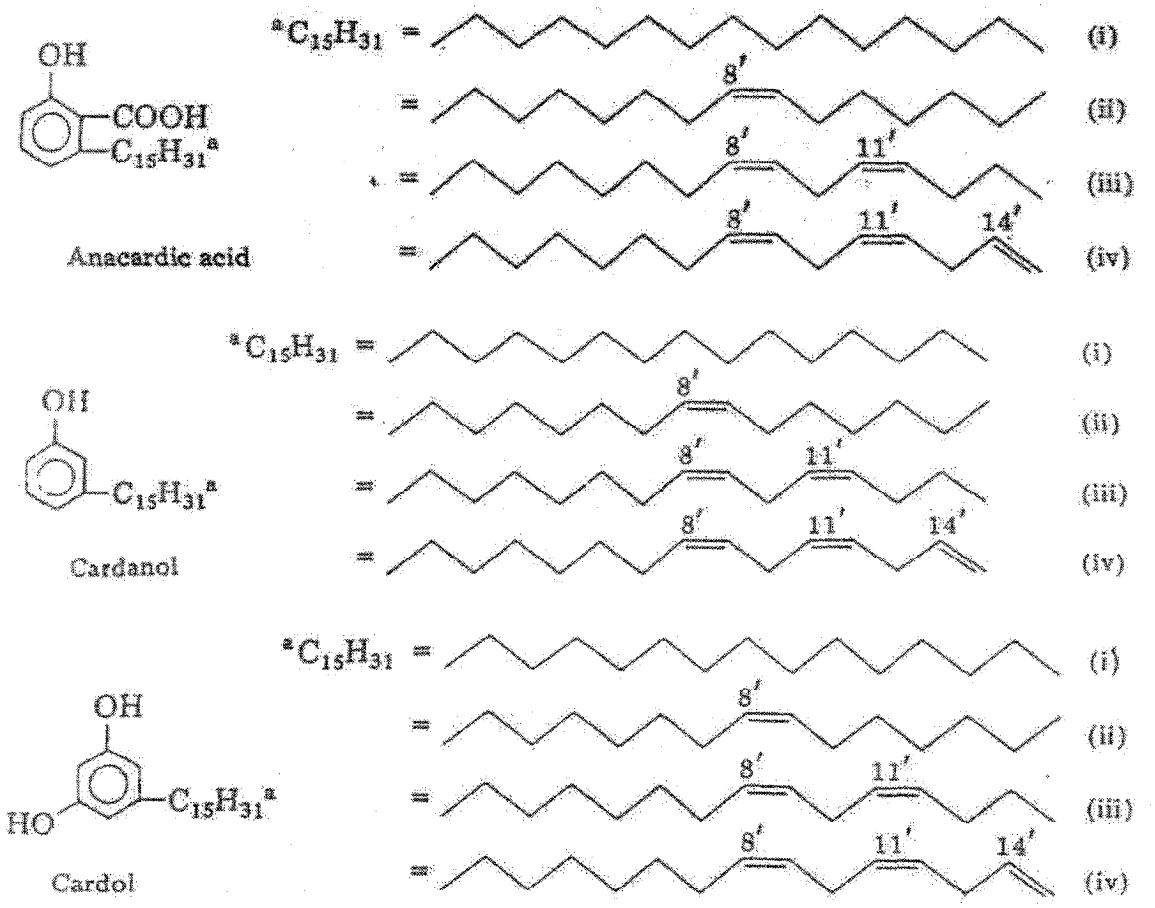
CNSL is traditionally obtained as a by-product during the process of removing the cashew kernel from the nut. The processes used are mainly hot-oil and roasting in which the CNSL oozes out from the shell.

The cashew tree is cultivated globally in tropical areas such as East Africa, South and Central America and the Far East. The world availability of CNSL is in the region of 50,000 tons/year.

### 1.1 Composition

Natural (i.e. cold, solvent extracted) CNSL contains approximately 70% anacardic acid (Fig 1), 18% cardol, and 5% cardanol, with the remainder being made up of other phenols and less polar substances. As can be seen in Figure 1, anacardic acid, cardanol and cardol consist of mixtures of components having various degrees of unsaturation in the alkyl side-chain.

Figure 1: Structures of Anacardic acid, Cardanol and Cardol




In technical (i.e. heat extracted) CNSL, the heating process leads to decarboxylation of the anacardic acid to form cardanol. Typically, the composition of technical CNSL is approximately 52% cardanol, 10% cardol, 30% polymeric material, with the remainder being made up of other substances.

The technical CNSL is often further processed by distillation at reduced pressure to remove the polymeric material. The composition of distilled technical CNSL is approximately 78% cardanol, 8% cardol, 2% polymeric material, < 1% 2-methyl cardanol, 2.3% heptadecyl homologue triene, 3.8% heptadecyl homologue diene and the remainder other homologous phenols.

Table 1 summarises the composition of typical batches of technical and distilled technical grades of CNSL.

Table 1: Composition of typical batches of Technical and Distilled CNSL

| | Cardanol | Cardanol monoene | Cardanol diene | Cardanol triene | Cardol diene | Cardol triene | Polymer | 2-methyl cardanol | C17 triene | C17 diene | Unidentified phenols |
|---|---|---|---|---|---|---|---|---|---|---|---|
| T-CNSL | 0.06 | 17.10 | 10.78 | 24.42 | 2.36 | 7.50 | 30.6 | | | | 5.83 |
| D-CNSL | - | 25.9 | 16.2 | 35.8 | 2.04 | 5.90 | 2.5 | 0.60 | 2.27 | 3.75 | 5.04 |
| AT-CNSL | 0.09 | 24.7 | 15.6 | 35.3 | 3.41 | 10.8 | - | | | | 8.42 |

T-CNSL = Technical grade, D-CNSL = Distilled grade, AT-CNSL = Technical grade component percentages adjusted for removal of polymer.

### 1.2 Commercial Applications

CNSL resins have been used extensively in the manufacture of friction- resistant components in applications such as brake and clutch linings. These resins are used as binders for friction ingredients and also as friction ingredients themselves in the form of fine dusts obtained from the completely cured resins.

CNSL-aldehyde condensation products and CNSL-based phenolic resins are used in applications such as surface coatings, adhesives, varnishes and paints. Various polyamines synthesised from CNSL or cardanol are used as curing agents for epoxy resins.

CNSL and its derivatives have been used as antioxidants, plasticisers and processing aids for rubber compounds and modifiers for plastic materials. Resins based on the reaction products of cardanol phenol and formaldehyde are used to improve the resistance of rubber articles to cracking and ozone. CNSL, cardanol and cardol are all used to provide oxidative resistance to sulfur- cured natural rubber products. Cardanol, CNSL or sulfurated CNSL is added to rubber gum stock or nitrile rubber to improve the processability, mechanical properties and resistance to crack and cut properties of the vulcanisates.

A number of products based on CNSL are used as antioxidants, stabilisers and demulsifiers for petroleum products. Metal xanthates of partially hydrogenated, sulfurised cardanol is used to lower the pour point of lubricating oils as well as acting as antioxidant and anticorrosive properties. Soluble metal derivatives of CNSL are used to improve the resistance to oxidation and sludge formation of lubricating oils. Oxidised CNSL and its derivatives are used as demulsifying agents for water in oil type petroleum emulsions.

### 1.3 Worker/consumer exposure

Only large industrial manufacturers use CNSL. There are no direct consumer applications and therefore no direct sales to the general publ ic. The most likely source of consumer exposure to CNSL is through contact with contaminated nuts, although reports of adverse effects arising from such contact appear to be rare.

Exposure of workers to CNSL during production is most likely to occur duri ng removal of the kernels from the nuts, after processing to remove the CNSL, especially in countries where the shelling has not been mechanised. Exposure to CNSL can lead to sensitisation and dermatitis. Workers in these countries are given some protect ion to exposure through the use of barrier creams.

Workers involved in the further processing the CNSL to manufacture commercial products are likely to have minimal exposure to the CNSL as it is expected that good industrial hygiene practices will be followed and personal protective equipment worn to minimise exposure.

## 2. Rationale for Selection of Compounds for testing

Distilled Technical CNSL has been selected as the most suitable substance for testing to fulfil the requirements of the HPV Challenge Program. This is because it is possible to obtain distilled CNSL to a more consistent specification than the Technical CNSL and the distilled grades are becoming more important industrially than the crude technical grade material. It is also believed that since all of the substances in CNSL are based on phenols having various degrees of unsaturation in the side chain, they meet the EPA's criterion of using the 'family approach', thus the toxicological properties of the Distilled CNSL will be representative of the properties of the Technical CNSL.

## 3. Review of Existing Data and Development of Test Plan

Cardolite Corporation has undertaken a comprehensive evaluation of all relevant data on the SIDS endpoints of concern for Cashew Nut Shell Liquid.

The availability of the data on the specific SIDS endpoints is summarized in Table 2. Table 2 also shows data gaps that will be filled by additional testing.

Table 2: Available Adequate Data and Proposed Testing on Cashew Nut Shell Liquid*

| CAS No. 8007-24-7 | Information Available? | GLP | OECD Study? | Other Study? | Estimation Method? | Acceptable? | SIDS Testing required? |
|---|---|---|---|---|---|---|---|
| | Y/N | Y/N | Y/N | Y/N | Y/N | Y/N | Y/N |
| **Physicochemical** | | | | | | | |
| Vapour Pressure | Y | - | - | - | Y | N | Y |
| Partition Coefficient (Kow) | N | - | - | - | - | - | Y |
| Water solubility | N | - | - | - | - | - | Y |
| **Environmental Fate & Pathway** | | | | | | | |
| Photodegradation | N | - | - | - | - | - | Y |
| Transport and distribution | N | - | - | - | - | - | Y |
| Biodegradation | Y | Y | Y | - | - | Y | N |
| **Ecotoxicology** | | | | | | | |
| Acute Fish | Y | - | - | - | Y | Y | N |
| Acute Daphnia | Y | - | - | - | Y | Y | N |
| Acute Algae | Y | - | - | - | Y | Y | N |
| **Toxicology** | | | | | | | |
| Acute Oral | N | - | - | - | - | - | N** |
| Repeat Dose toxicity | N | - | - | - | - | - | Y |
| Genetic toxicity – Gene mutation | Y | Y | Y | - | - | - | N |
| Genetic toxicity – Chromosome aberration | Y | Y | Y | - | - | - | N |
| Reproductive toxicity | N | - | - | - | - | - | Y |
| Developmental toxicity/teratogenicity | N | - | - | - | - | - | Y |

* No testing will be conducted for melting point, boiling point or hydrolysis.
** This endpoint will be filled using information from the OECD 422 range- finding study

### A. Evaluation of Existing Physicochemical Data and Proposed Testing

The basic physicochemical data required in the SIDS battery includes melting point, boiling point, vapor pressure, partition coefficient (Kow) and water solubility.

Cashew nut shell liquid (CNSL) meets the criteria for a Class 2 substance – it is a natural product that contains a number of chemical species and is of variable composition depending on its source and is, therefore, difficult to characterize and cannot be represented by a single chemical structural diagram. Due to this 'complex mixture' characteristic of CNSL, some physical property measurements do not give definitive results because the methodology used to determine these properties will fractionate or partition the substance into various components. Since the methodology will alter the actual sample composition, the results are likely to be erroneous or difficult to interpret.

#### 1. Melting Point

Melting point will not be determined, as the substance is a liquid under ambient conditions.

2. Boiling Point

A boiling point at ambient pressure has no significance, as the substance will be subject to thermal polymerization and decomposition before boiling. Accordingly, measurement of this property is inappropriate for this substance.

3. Vapor Pressure

The vapor pressure of CNSL will be determined using OECD Method 104.

4. Water solubility

Assuming adequate analytical sensitivity can be achieved, the water solubility of CNSL using OECD Method 105 will be determined, although estimation using EPIWIN predicts the solubility of cardanol, cardol and their structural analogs to be between 1 - $7x10^{-3}$ mg/L.

5. Partition Coefficient

The partition coefficient (i.e. Kow) for CNSL will be determined using OECD Method 107. It is likely that more than one Kow value, rather than a single value, will be generated when this endpoint is determined. This outcome reflects the complex nature of Class 2 mixtures.

**Summary of Physicochemical Properties Testing: The vapor pressure (OECD Method 104), water solubility (OECD method 105) and partition coefficient (OECD 107) of CNSL will be determined. Tests for melting point and boiling point are inapplicable to these substances.**

**B. Evaluation of Existing Environmental Fate Data and Proposed Testing**

The fate or behaviour of a chemical in the environment is determined by the reaction rates for the most important transformation (degradation) processes. The basic environmental fate data covered by the HPV Program include biodegradation, stability in water (hydrolysis as a function of pH), photodegradation and transport and distribution between environmental compartments.

1. Biodegradation

Biodegradability provides a measure for the potential of compounds to be degraded by microorganisms. Depending on the nature of the test material, several standard test methods are available to assess potential biodegradability.

Distilled CNSL has been shown to be biodegradable when tested using OECD Method 302D (96% degradation after 28 days) in a GLP study.

2. Hydrolysis

Hydrolysis as a function of pH is used to assess the stability of a substance in water. Hydrolysis is a reaction in which a water molecule (or hydroxide ion) substitutes for another atom or group of atoms present in an organic molecule. None of the major components of CNSL contain a functional group that would be susceptible to hydrolysis. Therefore, hydrolysis need not be measured.

In addition, low water solubility often limits the ability to determine hydrolysis as a function of pH. Estimation of the water solubility of the 2 main chemical components, and their analogs, of CNSL using E PIWIN v 3.04 predicts the solubility to be in the region of $1x10^{-3}$ mg/L. Therefore, these materials are expected to be stable in water and it would be unnecessary to attempt to measure the products of hydrolysis.

3. Photodegradation

The photodegradation of cardanol and cardol will be estimated using AOPWIN.

4. Transport and Distribution between Environmental Compartments

The transport and distribution between environmental compartments is intended to determine the ability of a chemical to move or partitio n in the environment. This property will be determined using a Level III Fugacity Model, for cardanol and cardol.

**Summary of Environmental Fate Testing: Biodegradation data exists for distilled CNSL. Photodegradation, and transport and distribution between environmental compartments will be estimated using AOPWIN and a Level III Fugacity Model, respectively.**

## C. Evaluation of Existing Ecotoxicity Data and Proposed Testing

The basic ecotoxicity data that are part of the HPV program include acute toxicity to fish, daphnia and algae. Predicted values for the 2 main chemical components, and their analogs, of CNSL have been obtained using ECOSAR v 0.99e[(2)]. These values predict that CNSL will be toxic in the aquatic environment, therefore it is unnecessary to generate experimental data. This is further supported by the predicted very low solubility of cardanol, cardol and their structural analogs, which are in the region of 1 – $7x10^{-3}$ mg/L, making analytical monitoring of the tests very difficult and making the tests themselves unlikely to determine any more useful or accurate estimates of toxicity than the estimations.

**Summary of Ecotoxicity Testing: The 2 main components of CNSL, and their analogs, are predicted to be acutely toxic to fish ($LC_{50}$ (96h) < $11x10^{-3}$ mg/l), daphnia ($LC_{50}$ (48h) < $66x10^{-3}$ mg/l) and algae ($EC_{50}$ (96h) < $1x10^{-3}$ mg/l).**

## D. Evaluation of Existing Human Health Effects Data and Proposed Testing

1. Acute Oral Toxicity

Acute oral toxicity investigates the effects of a single exposure to a relatively high dose of a substance. This test is conducted by administering the test material to animals (typically rats or mice) in a single gavage dose. Harmonized EPA testing guidelines (August 1998) set the limit dose for acute oral toxicity studies at 2000 mg/kg body weight. A compound that shows no effects at the limit dose is considered essentially non- toxic.

The range finding study to select doses for the OECD 422 study will be used to provide data on high -dose toxicity.

2. Repeat Dose Toxicity

Subchronic repeat dose toxicity studies are designed to evaluate the effect of repeated exposure to a chemical over a significant period of the life span of an animal. Typically, the exposure regimen in a subchronic study involves daily exposure (at least 5 consecutive days per week) for a period of between 28 – 90 days. The HPV program calls for a repeat dose test of at least 28 days. Repeat dose studies are designed to assess systemic toxicity, but the study protocol can be modified to incorporate evaluation of potential adverse reproductive and/or developmental effects.

The repeat dose toxicity of CNSL, combined with the reproductive/developmental toxicity screening test will be determined using OECD 422.

3. Genotoxicity

Genetic testing is conducted to det ermine the effects of substances on genetic material (i.e. DNA and chromosomes). The gene, which is composed of DNA, is the simplest functional genetic unit. Mutations of genes can occur spontaneously or as a consequence of exposure to chemicals or radiation. Genetic mutations are commonly measured in bacterial and mammalian cells, and the HPV program calls for completing both types of tests.

Distilled CNSL has been tested for potential genotoxicity in the Ames Salmonella assay (strains TA1535, TA1537, TA1538, TA98 and TA100), an in vitro chromosome aberration test in human lymphocytes, and an in vitro HGPRT forward mutation assay using a Chinese Hamster Ovary cell line. None of these test systems showed any indication of genotoxicity. All three studies were conducted under GLP.

4. Reproductive and Developmental Toxicity

Reproductive toxicity includes any adverse effect on fertility and reproduction, including effects on gonadal function, mating behaviour, conception and parturition. Developmental toxic ity is any adverse effect induced during the period of fetal development, including structural abnormalities, altered growth and post -partum development of the offspring.

The toxicity to reproduction aspect of the HPV Challenge Program can be met by conducting a reproductive/developmental toxicity screening test or adding a reproductive/developmental screening test to the repeat dose study (OECD 421 or 422, respectively).

As there is no repeat dose toxicity data for CNSL, the substance will be tested for repeat dose toxicity combined with the reproductive/developmental toxicity screening test according to OECD 422.

5. Skin Sensitisation

This non-SIDS endpoint has been evaluated using distilled CNSL in a Guinea pig maximisation test (OECD 406). The test substance produced a 70% (14/20) sensitisation rate and was classified as a strong sensitiser.

6. Oestrogenic Activity

This non-SIDS endpoint has been evaluated using two grades of distilled CNSL in a recombinant yeast screen assay. The two distillates showed no oestrogenic activity under the conditions of the test.

**Summary of Human Health Effects Testing: The repeat dose toxicity combined with the reproductive and developmental toxicity will be evaluated using OECD 422. The range finding study from this test will be used to provide information on the acute toxicity of the substance. Distilled CNSL has been tested and found negative in three *in vitro* genotoxicity assays, therefore no additional testing for this endpoint will be undertaken. Distilled CNSL has been shown to be a strong skin sensitiser in guinea pigs and to have no oestrogenic activity when tested in a recombinant yeast screen assay.**

## 4. Evaluation of Data for Quality and Acceptability

The collected data were reviewed for quality and acceptability following the general US EPA guidance (3) and the systematic approach described by Klimisch et al (4). These methods include consideration of the reliability, relevance and adequacy of the data in evaluating their usefulness for hazard assessment purposes. This scoring system was only applied to ecotoxicology and human health endpoint studies per EPA recommendation (5). The codification described by Klimisch specifies four categories of reliability for describing data adequacy. These are:

(1) Reliable without restriction: Includes studies or data complying with Good Laboratory Practice (GLP) procedures, or with valid and/or internationally accepted testing guidelines, or in which the test parameters are documented and comparable to these guidelines.

(2) Reliable with Restrictions: Includes studies or data in which test parameters are documented but vary slightly from testing guidelines.

(3) Not Reliable: Includes studies or data in which there are interferences, or that use non- relevant organisms or exposure routes, or which were carried out using unacceptable methods, or where documentation is insufficient.

(4) Not Assignable: Includes studies or data in which insufficient detail is reported to assign a rating, e.g. listed in abstracts or secondary literature.

## 7. References

1. EPIWIN v3.04. Meylan, W. & Howard, P. (1999), Syracuse Research Corporation, Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510

2. ECOSAR v0.99e. EPIWIN modelling program. Meylan, W. & Howard, P. (1999), Syracuse Research Corporation, Environmental Science Center, 6225 Running Ridge Road, North Syracuse, NY 13212-2510

3. USEPA (1998). Guidance for Meeting the SIDS Requirements (The SIDS Guide). Guidance for the HPV Challenge Program. Dated 11/2/98.

4. Klimisch, H.-J., et al (1997). A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data. Regul. Toxicol. Pharmacol. 25:1-5

5. USEPA (1999). Determining the Adequacy of Existing Data. Guidance for the HPV Challenge Program. Draft dated 2/10/99.